


# ITC titration

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Molecular engineering of metal coordination interactions for strong tough and fast-recovery hydrogels

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## Detailed protocol

Thank you for the attention on our paper.

For the sample preparation of XRFS samples, the HN-PH6 hydrogels were prepared and the coordination is completed as described in the experimental section. Then the hydrogels were dialyzed in the Tris buffer without  $Zn^{2+}$  ions to remove the unbinded ions. At last, the samples were lyophilized and measured using XRFS. As you know, the XRFS can remove the organic parts of the samples and the inorganic ion weights in the samples can be recorded. Besides, we can obtain the initial peptide weight inside the hydrogel samples by calculation based on the peptide reaction efficiency of the hydrogels. So we can finally calculate the weight ratio of peptide and  $Zn^{2+}$  ions in the hydrogels, and determine the binding stoichiometry of  $Zn^{2+}$  with PH6.

The ITC experiments were based on the PH6 molecule and  $Zn^{2+}$  ions, so it can only determine the binding affinity of PH6 peptide and  $Zn^{2+}$  ions. We use XRFS to determine the binding stoichiometry of  $Zn^{2+}$  with PH6 inside the hydrogels, which may directly affect the mechanical properties of the hydrogels.

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sun, W. , Wang, W. , Chen, B. and Cao, Y. (2021). ITC titration. Bio-protocol Preprint. [bio-protocol.org/prep1234](https://bio-protocol.org/prep1234).
2. Sun, W., Xue, B., Fan, Q., Tao, R., Wang, C., Wang, X., Li, Y., Qin, M., Wang, W., Chen, B. and Cao, Y.(2020). Molecular engineering of metal coordination interactions for strong tough and fast-recovery hydrogels . Science Advances 6(16). DOI: [10.1126/sciadv.aaz9531](https://doi.org/10.1126/sciadv.aaz9531)

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